GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 5/23/80

Project Title: Antiglaucoma Compounds From Cannabis Sativa

Project No: G-33-Q01

Project Director: Dr. Leon H. Zalkow

Sponsor: DHEW/PHS/NIH - National Eye Institute
Bethesda, MD 20014

Agreement Period: From 4/1/80 Until 3/31/81 (01 year)

Type Agreement: Grant No. 1 R01 EY03342-01

Amount: $94,200 New PHS Funds (G-33-Q01)
6,523 GIT Contribution (G-33-372)
$100,728 TOTAL

Reports Required: Interim Progress Reports with Continuation Applications
Terminal Progress Report upon Grant Expiration

Sponsor Contact Person (s):

Technical Matters

Program Official

Anita A. Suran, Ph.D
Extramural Program Director
Glaucoma Program
National Eye Institute
Bethesda, MD 20014

Contractual Matters
(thru OCA)

Grants Management Contact

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Extramural Services Branch
National Eye Institute
Bethesda, MD 20014

Defense Priority Rating: N/A

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

- Project Director
- Division Chief (EES)
- School/Laboratory Director
- Dean/Director—EES
- Accounting Office
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- Project Code (GTRI)
- Other C.E. Smith

B-17-22

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: 5/8/81

Project Title: Antiglaucoma Compounds From Cannabis Sativa

Project No: G-33-Q01

Project Director: Dr. Leon H. Zalkow

Sponsor: DHEW/PHS/NIH - National Eye Institute

Effective Termination Date: 3/31/81 (end of 01 year)

Clearance of Accounting Charges: ---

Grant/Contract Closeout Actions Remaining:

_ Final Invoice and Closing Documents
_ Final Fiscal Report
X Final Report of Inventions JWW: Request verification of need for annual rpt.
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
X Other Annual Report of Expenditures due by 6/30/81

NOTE: Follow-on project (02 year) is G-33-Q02

Assigned to: Chemistry (School/~~Laboratory~~)

COPIES TO:

Administrative Coordinator
Research Property Management
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Project File (OCA)
Other: _____

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	EY-03442-02
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT
ZALKOW, LEON H.	FROM: April 1, 1981 — THROUGH: March 31, 1982
NAME OF ORGANIZATION	
Georgia Institute of Technology	

TITLE (Repeat title shown in Item 1 on first page)
Antiglaucoma Compounds from Cannabis sativa

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1.(b) Publications:

H. M. Deutsch, K. Green and L. H. Zalkow, "Isolation of Ocular Hypotensive Agents from Cannabis sativa," J. Clin. Pharmacology, in press.

K. Green, L. H. Zalkow, H. M. Deutsch, M. E. Yablonski, N. Oliver, C. M. Symmonds and R. D. Elijah, "Ocular and Systemic Responses to Water Soluble Material Derived from Cannabis sativa," Current Eye Research, submitted.

Presentations:

H. M. Deutsch, L. H. Zalkow and K. Green, "Isolation of Hypotensive Agents from Cannabis sativa," 4th International Congress for Eye Research, New York, N.Y., Sept. 28-Oct. 3, 1980.

H. M. Deutsch, L. H. Zalkow and K. Green, "Isolation of Hypotensive Agents from Cannabis sativa," Fifth Pfizer Biomedical Research Symposium, Groton, Conn., Oct. 20-21, 1980.

K. Green, L. H.Zalkow, H. M. Deutsch, M. Yablonski, N. Oliver and C. Symonds, "Ocular and Systemic Responses to Water Soluble Material Derived from Cannabis sativa," to be presented at 53rd Annual ARVO Spring Meeting, Sarasota, Fla., April 26-May 1,1981.

2. Dr. L. Gelbaum, Research Scientist devoted 3 months and Dr. Maureen Gordon, Research Scientist, devoted 4 months time to this project during year 01. There were no other additions or deletions of professional personnel.

3. Progress Report

1. Objectives:

a. Total project.

(1)Isolate and characterize water soluble compound(s) from Cannabis sativa which on the basis of screening resul ts have been shown to lower intraocular pressure in test animals.

(2) Based on the results above, synthesize and/or modify the basic structural type to develop structural activity data and more effective compounds.

(3) Synthesize and screen water soluble derivatives of naturally occurring cannabinoids.

(4) Select and screen other plants as possible sources of compounds with IOP lowering activity.

b. Current Year.

(1) Isolate a pure compound with strong IOP lowering activity from active fractions.

(2) Begin the chemical characterization of this material.

(3) Study the mechanism of action of various active fractions in laboratory animals.

(4) Start the proecess of screening other plant materials.

* U. S. GOVERNMENT PRINTING OFFICE : 1974 584-258/3056

2. Studies During Current Year:

Most of the effort has been devoted to obtaining a pure compound with high IOP lowering activity. Initial efforts centered on conventional liquid chromatography and solvent extraction techniques. The solvent extraction technique (solid freeze dried aqueous extract, extracted with n-butanol, acetone, methanol and then the water soluble material freeze dried) produced material of high activity which was used in subsequent studies of the mechanism of its action in rabbits. However, little knowledge was gained about the nature of the material or its purity.

The chemical nature of the active compound(s) was partially revealed by dialysis experiments. The retentate, which represented about 20% of the crude fraction had high activity at 200µg/animal whereas the dialysate was inactive at the same dosage. Thus, it appeared that the active material was a high molecular polymer (MW > ~ 12,000 daltons). This was confirmed by gel filtration chromatography on Sephadex G-10, G-15, G-25 and G-50 on which the active material was eluted in the void volume. It was discovered that the ionic strength of the buffer was very important in these separations; ion strengths of at least 0.05M and, preferably, 0.30M were necessary to achieve good separations and avoid self aggregation. Excellent separations have been achieved on sephacryl S-200, which has a nominal exclusion limit of 250,000 daltons for globular proteins and 50,000 daltons for dextrans. Considerable technical problems were encountered in using this resin in regards to proper choice of columns, ionic strength and nature of buffer and proper flow rates. However, these problems have now all been worked out. The retentate from dialysis clearly shows two major bands; one sharp peak eluting near the void volume and a broad one centered about half way between the void volume and the included volume. All fractions showed strong activity at 200 µg/animal; however the void volume material was fully active at 1 µg/animal whereas none of the others showed activity below 5 µg/animal! Further screening of these fractions is in progress. It would appear that the high molecular weight material is nearly pure whereas the later eluting material is contaminated with the high molecular weight fraction and is polydisperse in molecular weight distribution (broad peak).

The best method of separation now appears to be to first chromatograph the retentate on DEAE cellulose using 0.05M tris + 0.25M Nace at pH 6.7. The first material eluted from the column (least acidic) is largely high molecular weight and has low UV absorpance at 254 nm. The next fraction is lower molecular weight with much stronger UV absorbance. Continued elution gives a very small fraction that is very dark in color, has very strong UV absorbance and is even lower in molecular weight distribution. Most of the IOP lowering activity is in the first fraction, but this has not been fully quantitated. The ion exchange procedure removes, irreversibly, a considerable amount (~ 35%) of very highly colored low molecular weight material and is now obviously the first method of purification before gel filtration. We plan on using affinity chromatography with concanavalin A dextrose now that these purer fractions are readily available.

Efforts to analyze and/or purify the material by gel electrophoresis have not met with much success to data. Conditions known to work with standard proteins do not work with these fractions. Thus, standard protein stains do not work at all, presumably due to very high carbohydrate content. Staining with periodate and Alcian Blue dye have shown some success and conditions are being optimized

for this procedure. Conditions for good separation e.g. strength of gel, % crosslinking, buffers and pH have not yet been worked out. When our new vertical slab gel unit is delivered, we expect to make rapid progress in this critical area of separation. We will also soon have access to a flat bed isoelectric focusing unit which might prove more fruitful than electrophoresis.

Because we have only recently been able to isolate relatively pure materials, only limited progress has been nade in chemical characterization. The crude retentate shows analysis for about 50% protein - 50% carbohydrate, although the results are somewhat variable due to highly colored lower molecular weight material. More highly purified fractions, such as the higher molecular weight material from gel filtration chromatography consistently show much lower protein analysis, in the range 10-20%. We are quite confident that the active material is a high carbohydrate containing glycoprotein, as material of this general type has been isolated from other strains of Cannabis and recently from other plants.

We expect that a major part of our effort in the next year will be in characterization of the pure active glycoprotein. Analysis of the number and type of sugars and amino acids present by standard chemical, spectroscopic, and chromatographic (including MS/GC) methods will be actively pursued. We also hope to apply recent advances in ^{13}C NMR spectroscopy to this problem since the School of Chemistry has recently acquired excellent facilities in this area (Bruker 300 MHz multinuclear Fourier Transform instrument). The carbohydrate-protein linkage and branching in the carbohydrate moiety will also be investigated by standard methods.

Our collaborator, Dr. K. Green, at the Medical College of Georgia has made extensive studies of the mechanism of action of several water soluble fractions in lowering the IOP of rabbits and monkeys. Most of this testing was done with the water soluble solvent extracted fraction. Intravenous administration produced a dose-related fall in intraocular pressure in both albino and pigmented rabbits with concentrations as low as 5 µg/animal sometimes being effective, but no response was found in monkeys. High concentrations (0.2 to 1 mg/animal) induced a hyptensive phase in intraocular pressure prior to the ocular hypotension; higher concentrations (2 or 5 mg/animal) also induced antidiuresis and general relaxation. Tachyphylaxis was found in repeated daily injections. Alpha and β-adrenergic antagonists caused some reduction of the hypertensive phase but had no effect on the hypotensive phase. Superior cervical ganglionectomy did not influence the time course of the intraocular pressure response. Indomethacin inhibited the hypertensive intraocular pressure phase but was ineffective against the hypotensive phase. Systemic blood pressure was unchanged following intravenous administration of 200 µg material/animal. Aqueous humor protein concentration was increased at both 1 and 6 hours after intravenous administration, becoming greater at the later time. Aqueous humor turnover rate was substantially reduced reaching a minimum 8.5 hours after administration. Topical administration was ineffective in eyes even when the epithelium was removed in rabbits with and without pretreatment with aspirin. Neither gastric nor suppository administration of large quantities (10 mg or greater) of material had any influence on intraocular pressure.

A number of other plants have been processed to give freeze dried water soluble extracts. Most of these have been totally inactive in lowering IOP when given intravenously in rabbits. However, at least two plants have shown activity (IOP fall of 50%) when tested at 200 µg/animal. These plants extracts have been

dialyzed and the retentates and dialysates are being screened for activity. We plan to vastly increase this phase of the project during the coming year. Many more plants will be screened and we plan to routinely dialyze and test any active material. Active samples will also be analyzed by analytical gel chromatography and/or gel electrophoresis.

We have not devoted any effort during the current year to modification of the basic structural type. When pure active material becomes available, extensive studies of its chemical nature will be undertaken. Studies of this type will be important in the next period. Enzymatic cleavage reactions will ge given the greatest preference, but selected chemical modifications will also be considered.

3. Significance to Health Problems

In view of the fact that glaucoma is the second leading cause of blindness in the United States, the importance of developing new and useful drugs in this area is obvious. The water soluble compounds isolated from marijuana have a more profound effect (lower IOP by 60%) than any other known compound at any dose level. Therefore the potential for benefit is indeed very great. If an easy to deliver, safe, effective drug can be developed from this work, it would be a very significant advance in glaucoma therapy. In any case, due to the potency of action, both in regards to the large decreases in IOP and the very small dose levels, this material is of fundamental interest to scientists interested in glaucoma and the mechanism of pressure regulation.

4. Research Goals During Coming Year

a. Continue established separation methods, with special emphasis on ion exchange and affinity chromatography, gel electrophoresis and isoelectric focusing, until a pure active material has been isolated.

b. Most of our effort will be geared towards the physical and chemical characterization of the above active fraction.

c. Selected chemical and biological transformations of the active material will be attempted in order to understand which portions of the molecule are necessary for IOP lowering activity, and to study its mechanism of action.

d. Screening of other plants will be increased to a considerable extent.

e. At least one water soluble derivative of a natural Cannabinoid will be made and screened for activity.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

3/10/81
Date

Principal Investigator